Preliminary data show that members of the FOXO family are expressed and functionally regulated in the developing myocardium. In vitro studies show that embryonic cardiac myocytes are responsive to growth factor stimulation, which results in the induction of the PI3K/AKT pathway, inactivation of FOXO proteins, and increased myocyte proliferation. These data support FOXO transcription factors as downstream effectors of PI3K/AKT signaling in cardiac myocytes and represent a novel approach to the investigation into the developmental regulation of cardiac myocyte proliferation. The generation of cardiac-specific FOXO transgenic mice and FOXO gene ablation studies using siRNA are currently in progress to further elucidate the role of FOXO factors in heart development.

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Mechanisms of caudal truncation in adrenocortical dysplasia (*acd*) mice

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Adrenocortical dysplasia (*acd*) is a spontaneous autosomal recessive mouse mutant with developmental defects in organs derived from the urogenital ridge: the kidneys, gonads, and adrenals. In addition, the *acd* mutation exhibits embryonic lethality on certain genetic strains, and analysis of *acd* mutant embryos reveals a striking embryologic phenotype that includes caudal truncation and axial skeletal patterning defects. We have previously characterized the *acd* mutation as a splicing defect in a gene (*Acd*) that encodes a novel component of the complex of telomere binding proteins that functions to maintain telomere integrity and regulate telomerase activity. Here, we report widespread expression of *Acd* mRNA in mouse embryos. We observed increased expression in the developing limb buds, tail, and neural tube, which resembles the embryonic expression pattern of the telomerase RNA component (*Terc*) gene and corresponds to the structural defects observed in *acd* mutant embryos. The function of ACD as a telomeric protein leads to the hypothesis that the mechanism leading to caudal truncation in *acd* mutant mice is via activation of p53, leading to apoptosis or cell cycle arrest. Preliminary studies reveal an increased number of TUNEL-positive cells in the caudal neural tube of *acd* embryos, but no gross differences in proliferation by PCNA immunohistochemistry, indicating that apoptosis is one mechanism leading to caudal truncation in *acd* mice. Further studies of proliferation and apoptosis in the caudal region of *acd* mutant embryos are currently in progress. This work was supported by NIH K08-HD42487 and the March of Dimes.

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Survival and differentiation of embryonic geniculate and trigeminal ganglia exposed at two stages to BMP-4 and noggin in vitro

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At rat embryonic day 13 (E13), nerve fibers from geniculate and trigeminal ganglia are within the early tongue. By E16, these ganglion cells innervate fungiform papillae and surrounding tongue epithelium, and thus are exposed to target-derived signaling factors. Bone morphogenetic protein 4 (BMP-4), known to be involved in neuron survival and differentiation, and its antagonist, noggin, are expressed in tongue by E13 and dramatically influence taste papilla development. To determine if these proteins affect the neurons that innervate tongue and papillae, we compared survival and neurite outgrowth in E13 and E16 geniculate and trigeminal ganglia explanted and cultured with exogenous BMP-4, noggin, or brain-derived neurotrophic factor (BDNF), a survival factor. Compared to geniculate ganglia exposed to BDNF, at E13 and E16, either BMP-4 or noggin resulted in a substantial decrease in neurons and neurite extension. The reduction was especially pronounced at E13. Survival and neurite extension also were decreased in E13 trigeminal neurons exposed to BMP-4 but were sustained by exposure to noggin. At E16, noggin continued to sustain a large population of trigeminal neurons, but BMP-4 was less effective at inhibiting survival. Thus, for the trigeminal ganglion a developmental shift in sensitivity to BMP-4 is evident. Geniculate and trigeminal ganglia display unique requirements for survival and differentiation factors during different developmental stages that perhaps relate to the heterogeneity of their respective neuronal populations.

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Differential regulation of male and female oligodendrocyte proliferation by hormones

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Sexual dimorphism has been established in gray matter regions of the brain and spinal cord. We recently showed that rodent white matter is sexually dimorphic (Cerghet et al., 2006). Moreover, exogenous hormones appear to regulate sexually dimorphic differences in turnover of oligodendrocytes (olgs) and myelin degradation in vivo. To investigate which hormones are involved, we treated enriched olg cultures from males and females grown in serum-free medium with different concentrations of estrogen (E2) and progesterone (P2), and counted the olgs after 4 days of exposure to hormones. The numbers of olgs increased 1.5× with 2.5 nM and 2× with 5

nM P2 in both males and females, with more olgs in females than males in untreated and 2.5 nM P2. This difference was eliminated at 5 nM concentration. In contrast to P2, E2 did not have any effects on the number of olgs at 10 and 50 nM E2. However, at 100 and 500 nM concentrations, E2 increased cell death in enriched olgs cultures in both males and females. We found that glial cultures have large numbers of cell clusters resembling neurospheres but they have never been studied. The cultures derived from females had more clusters compared to males. These clusters were positive for NG2, an olg precursor marker, and to nestin, a marker found mainly in neuroepithelial multipotential cells. The number of clusters increased about 2 to 4× in the cultures treated with 10 and 50 nM E2 compared to controls in both males and females. This study shows E2 principally affects olg progenitor proliferation and not differentiation whereas; P2 mostly affects the olg differentiation.

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Misregulation of oligodendrocyte number in *inxs*^{vu56} zebrafish larvae

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The number and distribution of oligodendrocytes, the myelinating cell type of the vertebrate central nervous system (CNS), is regulated such that target axons have uniform myelination and rapid conduction of nerve impulses. The mechanisms that ensure specification of sufficient numbers of oligodendrocytes and their distribution throughout the nervous system are poorly understood. They arise as proliferative and migratory oligodendrocyte progenitors cells (OPCs) that are fated to give rise to oligodendrocytes from *olig2*+ neural precursors in the ventral spinal cord that also produce motor neurons. Near the end of embryogenesis, OPCs stop dividing and start differentiating into myelinating oligodendrocytes. We are attempting to elucidate mechanisms that control oligodendrocyte number and distribution using transgenic zebrafish that express green fluorescent protein under the control of the *olig2* promoter in a screen to identify genes that are necessary for oligodendrocyte development. One mutation, *inxs*^{vu56}, was identified because it produces excess dorsally positioned OPCs in the spinal cord. Based on antibody studies of neuronal markers, we determined that the excess does not appear to be the result of fate switching or global expansion of the CNS. Therefore, we hypothesize that the excess oligodendrocytes of *inxs*^{vu56} mutant larvae results from elevated levels of OPC proliferation. We are currently testing this hypothesis by examining proliferation markers and using time-lapse photomicroscopy.

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Divergent proliferative roles for Pax3 and Pax7 in chick

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Pax3 and Pax7 are closely related members of the paired-box transcription factor family that are important in muscle development in the vertebrate embryo. Evidence from a number of labs suggests that Pax3 and Pax7 are functionally redundant. Our lab previously showed that signals, such as Wnt3a, that maintain or induce Pax3 and Pax7 expression in the neural tube and dermomyotome also cause an increase in proliferation. Based on these findings, we hypothesized that Pax3 and Pax7 may function as positive regulators of proliferation. To determine if Pax3 and Pax7 have proliferative roles, we stained chick somite explants for endogenous Pax3, Pax7, and a proliferation marker, phosphohistone H3. Contrary to our expectations, we found that Pax3-positive cells were more likely to stain for H3 than Pax3-negative cells, and conversely Pax7-positive cells were less likely to stain for H3 than Pax7-negative cells. Overexpression of Pax3 in ovo resulted in an increase in the number of proliferative cells as compared to control while overexpression of Pax7 resulted in a decrease in the number of proliferative cells as compared to control. Cumulatively, our results strongly suggest different functional roles for Pax3 and Pax7 with respect to proliferation. We can envision at least two scenarios to explain why our results differ from previous studies. The effects of Pax3 and Pax7 on proliferation may be dose dependent or may be affected by the presence of alternative transcripts. We were able to identify alternative transcripts of both Pax3 and Pax7 in somites. Studies to evaluate the functional significance of these transcripts are pending.

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